

Full Length Research Paper

Antibacterial, antifungal and phytochemical analysis of crude extracts from the leaves of *Ludwigia abyssinica* A. Rich. and *Ludwigia decurrens* Walter

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Ludwigia abyssinica and *Ludwigia decurrens* are two plant species of the genus *Ludwigia* used traditionally for the treatment of various skin, gastrointestinal, wound and bone joint disorders in Nigeria. The antibacterial and antifungal properties of extracts from the leaves of both plants against clinically important species of bacteria and fungi were examined. The two plant species produced activities with absolute similarity. The n-butanol extract was the most potent with maximum zone of inhibition (32.0 mm) followed by the ethyl acetate extract (12.0 to 31.0 mm) amongst other extracts (aqueous, dichloromethane and n-hexane). The n-butanol extract exhibited broad spectrum activity against all test bacteria and fungi and compared favourably with standard reference drugs – ampicillin, streptomycin and amphotericin B. The minimum inhibitory concentrations (MIC) exhibited by both n-butanol and ethyl acetate extracts against test bacteria species ranged between 0.625 to 5.0 mg ml⁻¹ and 1.25 to 5.0 mg ml⁻¹, respectively. The killing rate of the minimum bactericidal concentration (MBC) of n-butanol extract of *L. abyssinica* on *Escherichia coli* was about 99.3% in 120 min while it was about 98.2% for *Staphylococcus aureus*. The phytochemical screening of crude extracts from the leaves of *L. abyssinica* and *L. decurrens* revealed the presence of only alkaloids and tannins. This study establishes the effective ethnomedicinal use of these plants in the treatment of various infectious diseases. There is high potential for the exploitation of the plants for development of new, novel antimicrobial agents.

Key words: *Ludwigia abyssinica*, *Ludwigia decurrens*, Onagraceae, antibacterial, antifungal, phytochemical constituent.

INTRODUCTION

The use of plant parts by various human traditions in the preparation of herbal remedies is as old as human history (Cowan, 1999). These medicinal plants are used for the treatment of all kinds of ailments such as skin infections, sores, intestinal and respiratory conditions (Grabley and Thiercke, 1999; Nostro et al., 2000; Okigbo et al., 2005; Kubmarawa et al., 2007). There is a continuous increase in the development of resistance to the existing antimicrobial agents (Okeke et al., 2007). Secondary

plant metabolites with anti infective activities have attracted attention as natural products that can be used as substitutes for antibiotics resistant to pathogenic bacteria and fungi. They also provide basis for the development of new antimicrobials (Moreillion et al., 2005). Antimicrobial agents inhibit microorganisms by interfering with specific physiological character or metabolic functioning of microorganisms (Cowan, 1999). *Ludwigia species* are members of the family Onagraceae which are flowering plants belonging to the order Myrtales, comprising of 21 genera concentrated in the temperate regions but now well distributed in the tropical regions of the world (Chen et al., 1992). *Ludwigia species*

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are distinguished by absence of floral tube, (Eyde, 1981). They are predominantly aquatic herbs and shrubs growing in ditches, shallow marshy areas, riverbanks, ponds and slow moving streams. Many of the species have been observed to be very invasive in many parts of the world where they block water ways preventing the navigation of ships and boats (Chester and Holt, 1990; Zardini et al., 1991; McGregor et al., 1996). They are serious weeds in rice plantations in many parts of Africa and some species have been reported to exhibit allelopathic effects (Burkill, 1997; Dandelo et al., 2008, Sakpere et al., 2009).

Ludwigia species are however, useful as ornamental plants because of their showy flowers which are mostly yellow in colour which makes them to be on sale in many parts of Europe (Ruaux et al., 2009). They are also used as attachment hosts in the rearing of insects in the laboratory and some used for toxicity assessment (Wogu and Ugborogho, 2000). Leaves of *Ludwigia abyssinica* are used as vegetables in Zaria, Nigeria while leaves of *Ludwigia adscendens* are fed to livestock in Mali. The seeds of all *Ludwigia* species are very rich in oil (Burkill, 1997). The medicinal use of *Ludwigia* species dates back to decades and previous studies on several species have revealed it to be of value in traditional medicine. Leaves of some species are used as poultice in wound dressings and as remedy for dysentery. The leaf sap is also taken orally to stave off threatened abortion, flatulence and constipation (Kirtikar and Basu, 1987). Extract of leaves and stems of *L. adscendens* possesses a strong antimicrobial activity and is used against various skin diseases while the flower possesses anti-inflammatory activity (Selim, 2003). Roots of *Ludwigia hyssopifolia* is used as infusion for treatment of syphilis and for poulticing pimples while a decoction of it is used in diarrhoea, dysentery, flatulence, leucorrhoea and purgative vermifuge (Das et al., 2007). The leaves of *Ludwigia octovalvis* are known to have analgesic properties which make them useful in combination with other drug plants in the treatment of rheumatism (Burkill, 1997). The antibacterial activity of *Ludwigia suffruticosa* was established by Aliyu et al. (2008).

There has been virtually no report on the medicinal activity of members of the genus *Ludwigia* in Nigeria. This research was therefore carried out to investigate the antibacterial and antifungal activities as well as the phytochemical properties of two species of *Ludwigia* present in Nigeria - *L. abyssinica* and *L. decurrens*.

MATERIALS AND METHODS

Collection of plant materials

Leaves of *L. abyssinica* and *L. decurrens* were collected from the Obafemi Awolowo University, Ile Ife, Nigeria campus in March,

2010 and authenticated taxonomically at the herbarium of the Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria. Voucher specimens of *L. decurrens* (U.H.I 16191) and *L. abyssinica* (U.H.I 16192) were deposited at the herbarium for future reference.

Preparation of plant extracts

The leaves were air dried for 5 days and then powdered with a blender to give a total weight of 800 g. This was extracted exhaustively at room temperature with 50% aqueous-methanol for 72 h. The extracts were filtered using Whatman filter paper no 2 and then concentrated under reduced pressure. The crude extract obtained was thereafter suspended in distilled water and successively partitioned with n-hexane, dichloromethane, ethyl acetate and n-butanol in a sequential order of increasing polarity. The fractional solvent extracts obtained were concentrated to dryness on a rotary evaporator and then screened for their antimicrobial activities.

Phytochemical screening

The phytochemical screening of crude extracts from the 2 plant species were carried out to determine the presence of active secondary plant metabolites. The plant extracts were screened for the presence of carbohydrates, alkaloids, saponin, tannins, flavonoids, anthraquinones, phlobatannin and terpenoids according to established procedures (Trease and Evans, 1989; Sofowora, 1993; Jigna et al., 2006)

Selection of bacterial and fungal strains

The test organisms were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Bacillus subtilis*, *Bacillus stearothermophilus*, *Klebsiella pneumoniae*, *Clostridium sporogenes*, *Salmonella typhi* and *Serratia marcescens*. The fungal isolates used were *Aspergillus niger*, *Aspergillus flavus*, *Penicillium camemberti*, *Fusarium oxysporium*, *Trichophyton mentagrophytes* and *Candida albicans*.

Antibacterial activity

The antibacterial screening of extracts were carried out by using the agar-well diffusion method according to Lino and Degracious (2006) with slight modifications. The test bacteria were inoculated into tubes of peptone water and incubated at 37°C for 18 h before use. Each of the cultures was adjusted to 0.5 McFarland turbidity standard (10^8 CFU ml⁻¹). Two hundred microlitres of standardized cell suspensions were used to seed 20 ml sterile molten Mueller Hinton agar (FLUKA) in MacCartney bottles and these were poured into sterile plates after gentle swirling and allowed to set. A sterile cork borer (6mm diameter) was used to bore wells into which were introduced 0.2 ml of each of the crude extract at 20 mg ml⁻¹. Controls were set up by introducing the actual solvent used in constituting each extract into another well. Streptomycin at 1 mg ml and ampicillin at 10 µg ml⁻¹ were introduced into each of the remaining wells to serve as positive control. Each setup was prepared in triplicates for reproducibility. The culture plates were allowed to stand for pre-diffusion and were then incubated at 37°C for 24 h. Diameters of zones of inhibition around wells were measured and recorded as measure of antibacterial activity.

Antifungal activity

Fungal isolates were grown on Saboraud dextrose agar (FLUKA) at

Table 1. Phytochemical analysis of crude extracts from the leaves of *L. abyssinica* and *L. decurrens*.

Active principle	<i>L. abyssinica</i>	<i>L. decurrens</i>
Alkaloids	+	+
Flavonoids	-	-
Tannins	+	+
Saponin	-	-
Phlobatannins	-	-
Anthraquinones	-	-
Glycosides	-	-
Tepenioids	-	-

+ = Present, - = Absent.

25°C until they sporulated. The fungal spores were harvested into Sabouraud dextrose broth and standardized to an OD_{600nm} of 0.1 before use. Two hundred microlitres of this was used to seed 20 ml of Sabouraud dextrose agar in McCartney bottles and these were poured into sterile Petri plates after gentle swirling. Upon setting, wells were bored on the agar using sterile cork borer (6 mm diameter). One well was filled with the extract at concentration of 20 mg ml⁻¹, another with the solvent used for constitution and the remaining well filled with amphotericin B at a concentration of 1 mg ml⁻¹ as a positive control. Plates were allowed to stand for proper diffusion of extract into agar and then incubated at 25°C for 96 h. They were then examined for zones of inhibition (Igbinosa et al., 2009).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Two fold dilutions of each of the most active extracts, 20, 10, 5, 2.5, 1.25, 0.625 mg ml⁻¹ were prepared. The different extract concentrations were dispensed into each well bored into Mueller Hinton agar plates and appropriately labelled. The preparation was left to stand for diffusion of extract into agar before incubation at 37°C for 24 h for the bacterial strains. The MIC was defined as the lowest concentration able to inhibit any visible bacteria growth (Shahidi, 2004). The MBC of the ethyl acetate and n-butanol fractions were determined according to the method of Spencer and Spencer (2004) with slight modifications. Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared Mueller Hinton agar plates. These were then incubated at 37°C for 48 h. The MBC was taken as the concentration that did not show any growth on the new set of agar plates.

Determination of killing rate

Rate of killing studies of the most susceptible and least susceptible bacterial isolates when exposed to the MBC of the butanol fractions of *L. abyssinica* and *L. decurrens* were carried out. *E. coli* and *B. stearothermophilus* in case of *L. abyssinica* and *E. coli* and *S. aureus* in case of *L. decurrens*. Standardised inoculum (10⁶ CFU ml⁻¹) of test organism (0.5 ml) was mixed with 4.5 ml of MBC of n-butanol extract. The preparation was allowed to stand at room temperature and the rate of killing was determined over 3 h. At each 30 min interval, 0.1 ml of mixture was taken and plated out on sterile nutrient agar and the plates were incubated at 37°C for 24 h. Control plates containing organism suspension without extract were

also set up. The number of surviving colonies were counted and recorded against time (NCCLS, 1999).

RESULTS

The phytochemical analysis of the crude extracts of the leaves of both *L. abyssinica* and *L. decurrens* revealed the presence of only alkaloids and tannins of all the phytochemicals screened for. Flavonoids, saponin, anthraquinones, phlobatannins and carbohydrates were found to be absent (Table 1). The n-butanol extract exhibited the highest activity of all the 5 extracts followed by the ethyl acetate extract (Tables 2 and 5). Also the n-butanol and ethyl acetate extracts at concentration of 20 mg ml⁻¹ from both *L. abyssinica* and *L. decurrens* exhibited activity on all the test bacteria species producing zones of inhibition ranging from 12 to 32.0 mm. The dichloromethane extract from *L. abyssinica* also exhibited activity against all test bacteria species but to lesser extents (zone of inhibitions 10.0 to 22.0 mm).

Only n-butanol extracts from the two species had activity against all test fungi (zone of inhibition 30.0 mm on *A. niger*, *A. flavus*, *P. camemberti*, *F. oxysporium* and *T. mentagrophytes* but 6.0 mm on *C. albicans* in case of *L. abyssinica* and 4.0 mm on same organism in case of *L. decurrens*) (Tables 3 and 6). The extracts - dichloromethane, ethyl acetate and n-hexane were only active against *T. mentagrophytes* of all test fungi (Tables 3 and 6). The aqueous extract had no effect on any of the test bacteria or fungal species (Tables 2, 3, 5 and 6). Table 4 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for the active n-butanol and ethyl acetate extracts of *L. abyssinica*. The MIC of n-butanol extract was 0.625 mg ml⁻¹ on all test bacteria while the MIC of ethyl acetate ranged between 1.25 and 5.0 mg ml⁻¹ (Table 4). Also the MBCs of n-butanol (ranging between 0.625 to 5.0 mg ml⁻¹) were generally lesser than that of ethyl acetate (ranging between 1.25 to 10.0 mg ml⁻¹) Table 4.

Table 7 shows the MIC and MBC values for the active

Table 2. Antibacterial activities of crude extracts from the leaf of *L. abyssinica* against some species of bacteria.

Test bacteria	Mean zones of inhibition in mm*						
	AQ (20 mg/ml)	DCM (20 mg/ml)	ETAC (20 mg/ml)	HEX (20 mg/ml)	BUT (20 mg/ml)	AMP (10 µg/ml)	STR (1 mg/ml)
<i>S. aureus</i>	0.0	13.0	30.0	29.0	32.0	20.0	32.0
<i>E. faecalis</i>	0.0	22.0	31.0	0.0	32.0	14.0	32.0
<i>B. subtilis</i>	0.0	13.0	31.0	28.0	32.0	17.0	30.0
<i>E. coli</i>	0.0	17.0	31.0	0.0	32.0	20.0	21.0
<i>K. pneumonia</i>	0.0	17.0	30.0	23.0	32.0	17.0	30.0
<i>B. stearothermophilus</i>	0.0	10.0	30.0	22.0	32.0	20.0	34.0
<i>C. sporogenes</i>	0.0	12.0	28.0	25.0	32.0	17.0	32.0
<i>S. marcescens</i>	0.0	10.0	32.0	0.0	32.0	17.0	31.0
<i>S. typhi</i>	0.0	10.0	30.0	20.0	32.0	17.0	30.0
<i>P. aeruginosa</i>	0.0	10.0	28.0	0.0	32.0	14.0	28.0

mm* - Mean of three replicates, AQ – Aqueous, DCM – dichloromethane, ETAC – ethyl acetate, HEX – N-hexane, BUT – N- butanol, AMP – ampicillin, STR – streptomycin.

Table 3. Antifungal activities of crude extracts from the leaf of *L. abyssinica* against some species of fungi.

Test fungi	Mean zones of inhibition in mm*					
	AQ (20 mg/ml)	DCM (20 mg/ml)	ETAC (20 mg/ml)	HEX (20 mg/ml)	BUT (20 mg/ml)	AMPHOT B (10 µg/ml)
<i>A. niger</i>	0.0	0.0	0.0	0.0	30.0	24.0
<i>A. flavus</i>	0.0	0.0	0.0	0.0	30.0	25.0
<i>P. camemberti</i>	0.0	0.0	0.0	0.0	30.0	22.0
<i>F. oxysporium</i>	0.0	0.0	0.0	0.0	30.0	24.0
<i>T. mentagrophytes</i>	0.0	30.0	16.0	24.0	30.0	22.0
<i>C. albicans</i>	0.0	0.0	0.0	0.0	6.0	15.0

mm* - Mean of three replicates, AQ – Aqueous, DCM – dichloromethane, ETAC – ethyl acetate, HEX – N-hexane, BUT – N- butanol, AMPHOT B – amphotericin B.

Table 4. MIC and MBC of active extracts of *L. abyssinica*.

Test bacteria	Average MIC / MBC of the extracts			
	Ethyl acetate		N-Butanol	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>S. aureus</i>	2.5	5.0	0.625	1.25
<i>E. faecalis</i>	1.25	2.5	0.625	1.25
<i>B. subtilis</i>	1.25	5.0	0.625	2.5
<i>E. coli</i>	2.5	5.0	0.625	0.625
<i>K. pneumoniae</i>	1.25	5.0	0.625	2.5
<i>B. stearothermophilus</i>	2.5	20.0	0.625	5.0
<i>C. sporogenes</i>	5.0	20.0	0.625	2.5
<i>S. marcescens</i>	1.25	1.25	0.625	1.25
<i>S. typhi</i>	1.25	5.0	0.625	1.25
<i>P. aeruginosa</i>	1.25	10.0	0.625	2.5

Table 5. Antibacterial activities of crude extracts from the leaf of *L. decurrens* against some species of bacteria.

Test bacteria	Mean zones of inhibition in mm*						
	AQ (20 mg/ml)	DCM (20 mg/ml)	ETAC (20 mg/ml)	HEX (20 mg/ml)	BUT (20 mg/ml)	AMP (10 µg/ml)	STR (1 mg/ml)
<i>S. aureus</i>	0.0	14.0	12.0	24.0	32.0	20.0	32
<i>E. faecalis</i>	0.0	0.0	13.0	0.0	32.0	14.0	32
<i>B. subtilis</i>	0.0	13.0	16.0	25.0	32.0	17.0	30
<i>E. coli</i>	0.0	0.0	13.0	0.0	32.0	20.0	21
<i>K. pneumonia</i>	0.0	14.0	14.0	26.0	32.0	17.0	30
<i>B. stearothermophilus</i>	0.0	13.0	16.0	0.0	32.0	20.0	34
<i>C. sporogenes</i>	0.0	14.0	15.0	26.0	32.0	17.0	32
<i>S. marcescens</i>	0.0	0.0	15.0	0.0	32.0	17.0	31
<i>S. typhi</i>	0.0	0.0	16.0	0.0	32.0	17.0	30
<i>P. aeruginosa</i>	0.0	13.0	16.0	24.0	32.0	14.0	28

mm* - Mean of three replicates, AQ – Aqueous, DCM – dichloromethane, ETAC – ethyl acetate, HEX – N-hexane, BUT – N- butanol, AMP – ampicillin, STR – streptomycin.

Table 6. Antifungal activities of crude extracts from the leaf of *L. decurrens* against some species of bacteria.

Test fungi	Mean zones of inhibition in mm*					
	AQ (20 mg/ml)	DCM (20 mg/ml)	ETAC (20 mg/ml)	HEX (20 mg/ml)	BUT (20 mg/ml)	AMPHOT B (10 µg/ml)
<i>A. niger</i>	0.0	0.0	0.0	0.0	30.0	25.0
<i>A. flavus</i>	0.0	0.0	0.0	0.0	30.0	24.0
<i>P. camemberti</i>	0.0	0.0	0.0	0.0	30.0	26.0
<i>F. oxysporium</i>	0.0	0.0	0.0	0.0	30.0	24.0
<i>T. mentagrophytes</i>	0.0	30.0	26.0	18.0	30.0	27.0
<i>C. albicans</i>	0.0	0.0	0.0	0.0	4.0	20.0

mm* - Mean of three replicates, AQ – Aqueous, DCM – dichloromethane, ETAC – ethyl acetate, HEX – N-hexane, BUT – N- butanol, AMPHOT B – amphotericin B.

n-butanol and ethyl acetate extracts of *L. decurrens*. The ethyl acetate extract was active against all test bacteria species with *C. sporogenes* and *S. marcescens* being the most susceptible (MIC = 1.25 mg ml⁻¹) and *S. aureus* and *K. pneumoniae* the least susceptible. The n-butanol extract was least inhibitory against *C. sporogenes*, *Salmonella typhi* and *P. aeruginosa* (MIC = 2.5 mg ml⁻¹) while it was most inhibitory against all other test organisms at MIC = 1.25 mg ml⁻¹. The MBC values of the n-butanol extract were generally lesser than that of ethyl acetate. In case of ethyl acetate, *B. stearothermophilus* had highest MBC (20 mg ml⁻¹) while *S. typhi* had the least MBC (2.5 mg ml⁻¹). In case on n-butanol, *E. coli*, *K. pneumoniae* and *S. marcescens* had least MBC (1.25 mg ml⁻¹) while *S. aureus*, *E. faecalis* and *P. aeruginosa* had highest MBC (5.0 mg ml⁻¹) (Table 7). The killing rate of the most inhibited test organism – *E. coli* was high with no surviving cells observed after 210 min of exposure to

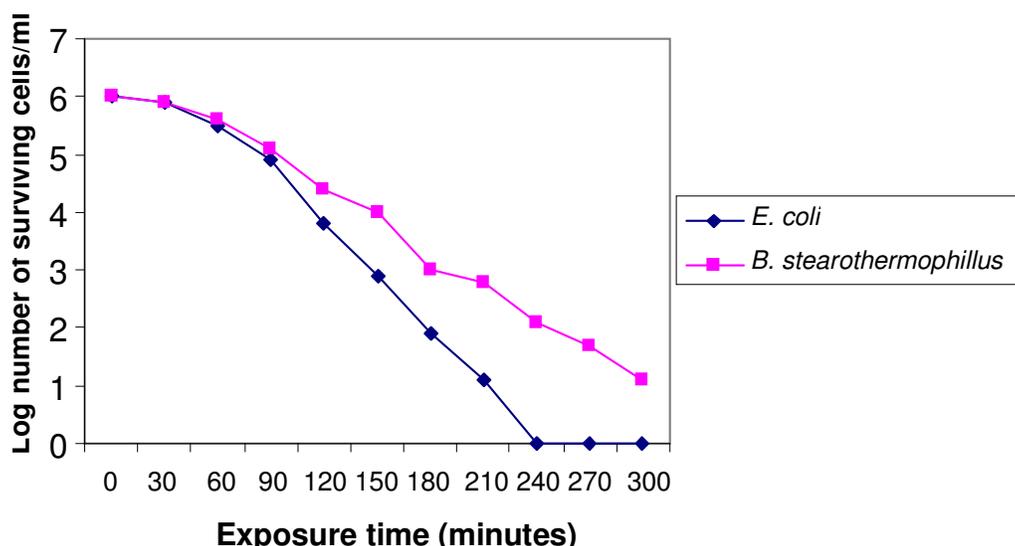
MBC of n-butanol extract of *L. abyssinica* whereas the least inhibited organism – *B. stearothermophilus* was still having surviving cells after 300 min of exposure to same treatment (Figure 1). The killing rate of the most inhibited test organism – *E. coli* was high with no surviving cells observed after 240 min of exposure to MBC of n-butanol extract of *L. decurrens* whereas the least inhibited organism – *S. aureus* was still having surviving cells after 300 min of exposure to same treatment (Figure 2).

DISCUSSION

The increasing trend of resistance to the antibiotics in current use has drawn the attention of researchers to natural alternative treatments of bacterial infections as potential sources of new, novel antimicrobial agents. This study indicated that *L. abyssinica* and *L. decurrens* which

Table 7. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of active extracts of *L. abyssinica*.

Test bacteria	Average MIC / MBC of the extracts			
	Ethyl acetate		N-Butanol	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>S. aureus</i>	5.0	5.0	1.25	5.0
<i>E. faecalis</i>	2.5	5.0	1.25	5.0
<i>B. subtilis</i>	2.5	10.0	1.25	2.5
<i>E. coli</i>	2.5	10.0	1.25	1.25
<i>K. pneumoniae</i>	5.0	10.0	1.25	1.25
<i>B. stearothermophilus</i>	2.5	20.0	1.25	2.5
<i>C. sporogenes</i>	1.25	10.0	2.5	2.5
<i>S. marcescens</i>	1.25	10.0	1.25	1.25
<i>S. typhi</i>	2.5	2.5	2.5	2.5
<i>P. aeruginosa</i>	2.5	5.0	2.5	5.0

**Figure 1.** Rate of killing of most susceptible test organism and least susceptible organism by MBC of n-butanol extract of *L. abyssinica*.

are used as medicinal plants in various regions of the world are active against a broad spectrum of bacterial and fungal species. Gram negative bacteria are reported to be resistant against most antibacterial agents as a result of the more complicated nature of their cell wall compared to Gram positive bacteria (Akinyemi et al., 2005; Ndukwe et al., 2007; Goyal et al., 2008). However, the two species were found to be active against the two groups of bacteria underlining their ethnomedicinal use for treatment of various infectious diseases (Kirtikar and Basu, 1987; Ahmed et al., 2004; Das et al., 2007; Aliyu et al., 2008).

The two species under study exhibited absolute similarity in their antibacterial, antifungal and

phytochemical properties though *L. abyssinica* with MIC 0.625 mg ml^{-1} was more potent than *L. decurrens* with MIC $1.25 \text{ to } 2.5 \text{ mg ml}^{-1}$. This finding established similarity in the active principles of most species of *Ludwigia* and similarity of their traditional use for various medicinal purposes. Aliyu et al. (2008) found the ethyl acetate and n-butanol extracts as the most active extracts of *L. suffruticosa* while Das et al. (2007) established the antimicrobial activity of ethyl acetate extract of *L. hyssopifolia* Linn. Overall the test organisms were most sensitive to the n-butanol extract followed by the ethyl acetate extract. Moreover, the n-butanol extract was highly effective against Gram positive and negative bacteria as well as all fungal species while the ethyl

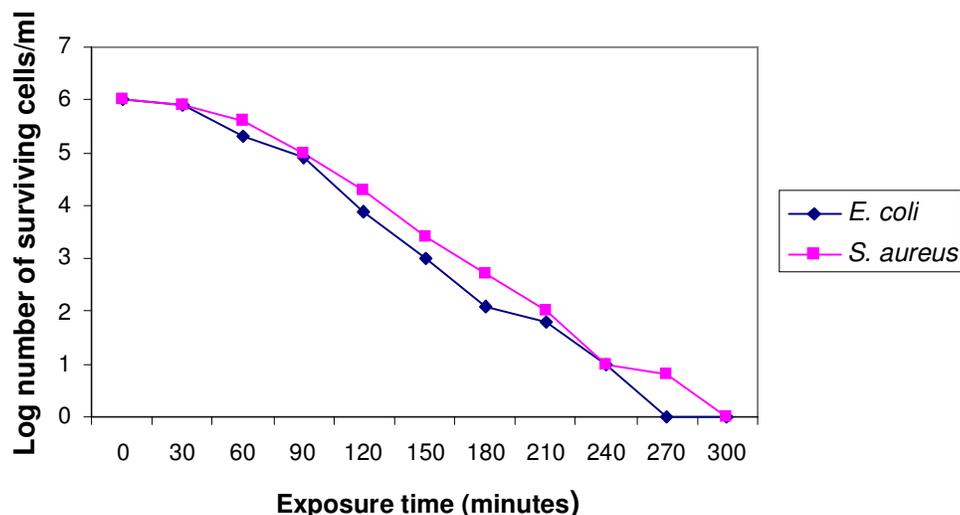


Figure 2. Rate of killing of most susceptible test organism and least susceptible organism by MBC of n-butanol extract of *L. decurrens*.

acetate extract only had high antimicrobial activities against all test bacteria species but not against fungal species except on *T. mentagrophytes*. The other extracts - dichloromethane and n-hexane only exhibited limited activities while the aqueous extract showed no activity against any of the test organism. This result shows that n-butanol and ethyl acetate are the most efficient extracting solvents for active principles of these plants.

The antibacterial activities of n-butanol and ethyl acetate extracts from both species compared well with that of standard antibiotics – ampicillin ($10 \mu\text{g ml}^{-1}$) and streptomycin (1 mg ml^{-1}). The n-butanol extracts also produced high activity against the test fungal species comparable to the standard antifungal agent – amphotericin B ($10 \mu\text{g ml}^{-1}$). The n-butanol therefore indicates a potential source of antimicrobial agent which should be studied further while the ethyl acetate extract also contain novel bioactive compounds which might be present in low concentrations but which on further purification might demonstrate equally high antimicrobial activities. A reduction in microbial population to 99% of initial population of the organism within the shortest period is the generally accepted definition of bactericidal activity in antibiotics (Pankey and Sahath, 2004). The killing rate of the MBC of the n-butanol extract of *L. abyssinica* on *E. coli* was about 99.3% in 120 min while it was 97.5% for *B. stearothermophilus*. For *L. decurrens*, the killing rate of MBC of the n-butanol extract on *E. coli* was about 99.3% while it was about 98.2% for *S. aureus*.

Investigations on the phytochemical constituents of crude extracts of *L. abyssinica* and *L. decurrens* revealed the presence of only alkaloids and tannins. These compounds are described as potent biologically active compounds found in medicinal plants parts which are

precursors for clinically useful drugs (Sofowora, 1993). The potency of medicinal plants is attributed to the action of the phytochemical constituents (Balandrin et al., 1985). These are actually produced by plants as secondary metabolites in response to environmental pressure or as a defense mechanism to animal or plant diseases. Several biological effects are ascribed to these compounds. For instance, many physiological activities such as stimulation of phagocytic cells, host mediated tumour activity and wide range of anti-infection actions are assigned to tannins (Okwu and Okwu, 2004). They also provide soothing relief; regenerates skin, produce anti-inflammatory effect and act as diuretics. Their antimicrobial effects are exerted through mechanisms such as membrane disruption, binding to proteins and adhesions, enzyme inhibition, substrate deprivation and metal ion complexation (Cowan, 1999). Medicinal plants having tannins as the main components are astringent in nature and are used for treatment of intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). Alkaloids exhibit marked physiological effects when administered to animals and hence their wide use in medicine for development of drugs (Harborne, 1973; Okwu, 2005). They produce analgesic, antispasmodic and bactericidal effects (Stray, 1998). They interfere with processes such as deoxyribonucleic (DNA) replication and ribonucleic acid (RNA) transcription which are vital to microorganisms. Other mechanisms are disruptions of protein synthesis, stability of biomembranes and metabolically important enzymes (Cowan, 1999). The broad spectrum activity exhibited by extracts from the two species of plants studied establishes the scientific basis for their use as ethnomedicine and their potential for use for development of novel antimicrobial agents effective

for treatment of microbial infectious diseases. Their usefulness in the formulation of antiseptics and disinfectants is also recommended if the active principles can be isolated and purified. Further investigations of the plants for the isolation, purification and characterization of the active principles are on going.

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